3.0 RESULTS

3.1 *Receiving Water and Fecal Source Samples*

Table 1. gives the number of samples, dates of sampling, and whether a base or storm flow was sampled for each stream location. The four primary locations—CVLS01, CVLS03, CVLS07, and CVLS08—are described above. Eight of the samples taken at each site were used for MST except CVLS03 where seven samples and one from a tributary site, CVLS03a, were used. Selected samples were used from those taken at CVLS04, CVLS05, and CVLS06.

Table 2. gives the number of samples, dates of sampling, and locations (also Figure 2.) for each source type. Each sample was collected from a unique animal. Septic tank samples may be from individual tanks or from different locations within a single tank. This is indicated in the table. Domestic animals sampled included chickens (3), cows (10), dogs (8), goats (2), horses (20), llamas (5), and pigs (5). Wild animals sampled were deer (2), ducks (2), and fish (10). Septic tanks sampled represent single family residences (3), businesses (1), and public facilities (2).

3.2 *Fecal Coliform Enumeration*

All base flow fecal coliform concentrations were less than 100 CFU/100 mL for CVLS01 through CVLS06. The exception is CVLS03a. This site is located on a tributary of Little Soos Creek upstream from CVLS03 (Figure 2.) and was only sampled once. The tributary is a small stream that drains a number of pastures and properties served by septic systems just prior to its confluence with Little Soos Creek. The base flow fecal coliform count at CVLS03a on 18 May 1994 was 630 CFU/100 mL. Base flow counts for the lower reach ranged from 22 to 200 CFU/100 mL (geometric mean of 59 CFU/100 mL) for CVLS07 and from 40 to 520 CFU/100 mL (geometric mean of 115 CFU/100 mL) for CVLS08.

Storm flow fecal coliform counts were greater than base flow counts on average and were highest at all sites for the 18 February 1995 storm. This was an intense storm occurring after a long period of variable dry and wet weather with little significant rainfall. It occurred during a time of year when the water table is typically high. These factors can increase the potential for accumulated surface and subsurface pollutants to be washed into the stream in a relatively short period of time. The counts for this date increased significantly from upstream to downstream. Results ranged from 160 CFU/100 mL at CVLS01 to 410 and 400 CFU/100 mL at CVLS04 and CVLS05 respectively. Concentrations downstream were 4800 CFU/100 mL at CVLS06 and 3100 CFU/100 mL at CVLS07. The counts obtained for CVLS08 and its duplicate were 3000 and 5000 CFU/100 mL respectively. Since a stream is not necessarily completely mixed, it is not

Table 1. Summary of water data and fecal coliform bacteria concentrations in Little Soos Creek.

Sample Location[1]	Sample Number[2]	Sampling Date	Concentration CFU[3]/100 mL	Number of Isolates	Number of Ribotypes	Totals for Location
CVLS01	CVLS01-02B	16-Sep-93	3	2	2	# isolates = 71
	CVLS01-04S	21-Nov-93	3	8	4	
	CVLS01-05B	16-Nov-93	9	10	4	# unique ribotypes = 27
	CVLS01-07S	13-Feb-94	60	14	5	
	CVLS01-08B	18-Jan-94	0	8	1	
	CVLS01-10S	18-Feb-95	160	19	8	
	CVLS01-11B	30-Mar-94	2	2	2	
	CVLS01-14B	18-May-94	8	8	5	
CVLS03	CVLS03-01B	16-Sep-93	34	16	10	# isolates = 114(111)[4]
	CVLS03-02S	21-Nov-93	22	16	6	
	CVLS03-03B	16-Nov-93	23	16	11	# unique ribotypes = 53
	CVLS03-04S	13-Feb-94	60	16	11	
	CVLS03-05B	18-Jan-94	60	16	12	
	CVLS03-06S	18-Feb-95	230	19(16)[4]	9	
	CVLS03-07B	30-Mar-94	60	15	6	
CVLS03a	CVLS03a-01B	18-May-94	630	13	8	# isolates = 13
						# unique ribotypes = 8
CVLS04	CVLS04-04S	18-Feb-95	410	20	12	# isolates = 20
						# unique ribotypes = 12
CVLS05	CVLS05-03B	18-Jan-94	80	8	5	# isolates = 26
	CVLS05-04S	18-Feb-95	400	18	10	
						# unique ribotypes = 13
CVLS06	CVLS06-07S	13-Feb-94	360	16	11	# isolates = 53(32)
	CVLS06-10S	18-Feb-95	4800	37(16)	10	
						# unique ribotypes = 20
CVLS07	CVLS07-01B	16-Sep-93	200	15	6	# isolates = 152(128)
	CVLS07-02S	21-Nov-93	31	16	9	
	CVLS07-03B	16-Nov-93	59	16	9	# unique ribotypes = 64
	CVLS07-04S	13-Feb-94	290	14	7	
	CVLS07-05B	18-Jan-94	22	16	14	
	CVLS07-06S	18-Feb-95	3100	40(16)	12	
	CVLS07-07B	30-Mar-94	26	21	13	
	CVLS07-09B	18-May-94	110	14	10	
CVLS08	CVLS08-02B	16-Sep-93	130	15	9	# isolates = 153(126)
	CVLS08-04S	21-Nov-93	700	16	8	
	CVLS08-05B	16-Nov-93	470	16	13	# unique ribotypes = 56
	CVLS08-07S	13-Feb-94	120	16	6	
	CVLS08-08B	18-Jan-94	40	16	12	
	CVLS08-10S	18-Feb-95	3000	43(16)	9	
	CVLS08-11B	30-Mar-94	43	16	11	
	CVLS08-14B	18-May-94	110	15	6	
CVDUPS (duplicate of CVLS08)	CVDUPS-02B	16-Sep-93	160	16	9	# isolates = 62
	CVDUPS-04S	21-Nov-93	300	16	11	
	CVDUPS-05B	16-Nov-93	520	16	14	# unique ribotypes = 36
	CVDUPS-08B	18-Jan-94	55	15	10	
	CVDUPS-14B	18-May-94	160	These samples were not used for MST. The fecal coliform counts were used in calculating the geometric mean value given in text.		
	CVDUPS-10S	18-Feb-95	5000			
All Samples						# isolates = 664(589)
						# unique ribotypes = 171

[1] see Figure 2. [2] B = base; S = storm [3] CFU = colony forming units [4] ( ) indicates adjusted total. See text.

Table 2. Summary of source sample data from Little Soos Creek watershed and vicinity.

Source Type	Sample Number	Sampling Date	Sample Location[1]	Number of Isolates[2]	Number of Ribotypes	Totals for Source Type
chicken	CH-01	28-Sep-93	CVLS05	14	6	# isolates = 40
	CH-02	9-Aug-94	vicinity	13	7	
	CH-03	9-Aug-94	vicinity	12	6	# unique ribotypes = 17
						matches w/ water ribotypes = 18%
cow	CO-01	13-Apr-94	CVLS03a	16	4	# isolates = 189
	CO-02	13-Apr-94	CVLS03a	16	6	
	CO-03	9-Aug-94	vicinity	16	3	# unique ribotypes = 39
	CO-04	18-Nov-94	CVLS06/07	32	5	matches w/ water ribotypes = 33%
	CO-05	18-Nov-94	CVLS07/08	32	5	
	CO-06	23-Feb-95	vicinity	16	7	
	CO-07	23-Feb-95	vicinity	16	6	
	CO-08	23-Feb-95	vicinity	16	5	
	CO-09	23-Feb-95	vicinity	16	8	
	CO-10	23-Feb-95	vicinity	14	2	
deer	DE-01	13-Apr-94	CVLS01	0	--	# isolates = 7
	DE-02	13-Apr-94	CVLS01	7	2	
						# unique ribotypes = 2
						matches w/ water ribotypes = 0%
dog	DO-01	28-Sep-93	CVLS04	18	4	# isolates = 130
	DO-02	28-Sep-93	CVLS05	2	1	
	DO-03	13-Apr-94	CVLS04	0	--	# unique ribotypes = 22
	DO-04	13-Apr-94	CVLS03	16	4	matches w/ water ribotypes = 32%
	DO-05	9-Aug-94	vicinity	10	4	
	DO-06	18-Nov-94	CVLS06/07	32	11	
	DO-07	24-Jan-95	CVLS05/06	32	2	
	DO-08	24-Jan-95	CVLS05/06	18	2	
duck	DU-01	30-Oct-94	CVLS08	16	8	# isolates = 31
	DU-02	30-Oct-94	CVLS08	14	6	
						# unique ribotypes = 12
						matches w/ water ribotypes = 0%
fish	FI-01	17-Nov-93	CVLS07/08	0	--	# isolates[2] = 0
	FI-02	15-Dec-93	CVLS07/08	0	--	
	FI-03	15-Dec-93	CVLS07/08	0	--	# unique ribotypes = 0
	FI-04	15-Dec-93	CVLS07/08	0	--	matches w/ water ribotypes = 0%
	FI-05	15-Dec-93	CVLS07/08	0	--	
	FI-06	15-Dec-93	CVLS07/08	0	--	
	FI-07	15-Dec-93	CVLS07/08	0	--	
	FI-08	15-Dec-93	CVLS07/08	0	--	
	FI-09	15-Dec-93	CVLS07/08	0	--	
	FI-10	15-Dec-93	CVLS07/08	0	--	
goat	GO-01	9-Aug-94	vicinity	7	1	# isolates = 22
	GO-02	23-Feb-95	vicinity	15	1	
						# unique ribotypes = 2
						matches w/ water ribotypes = 50%

[1] see Figure 2. [2] A "0" value indicates *E. coli* could not be isolated from the sample.

Table 2. Summary of source sample data (continued).

Source Type	Sample Number	Sampling Date	Sample Location[1]	Number of Isolates[2]	Number of Ribotypes	Totals for Source Type
horse	HO-01	28-Sep-93	CVLS02/03	1	1	# isolates = 260
	HO-02	28-Sep-93	CVLS03	16	2	
	HO-03	28-Sep-93	CVLS05	14	6	# unique ribotypes = 39
	HO-04	13-Apr-94	CVLS04/05	1	1	matches w/ water ribotypes = 36%
	HO-05	13-Apr-94	CVLS02/03	6	4	
	HO-06	9-Aug-94	vicinity	7	2	
	HO-07	14-Sep-94	vicinity	5	3	
	HO-08	14-Sep-94	vicinity	16	3	
	HO-09	14-Sep-94	vicinity	16	7	
	HO-10	30-Oct-94	CVLS08	3	1	
	HO-11	30-Oct-94	CVLS08	19	3	
	HO-12	30-Oct-94	CVLS08	4	1	
	HO-13	30-Oct-94	CVLS08	10	2	
	HO-14	30-Oct-94	CVLS08	4	3	
	HO-15	30-Oct-94	CVLS08	28	4	
	HO-16	24-Jan-95	vicinity	36	2	
	HO-17	24-Jan-95	vicinity	28	4	
	HO-18	23-Feb-95	vicinity	15	2	
	HO-19	23-Feb-95	vicinity	16	6	
	HO-20	23-Feb-95	vicinity	15	4	
llama	LL-01	23-Feb-95	vicinity	9	4	# isolates = 69
	LL-02	23-Feb-95	vicinity	12	4	
	LL-03	23-Feb-95	vicinity	16	4	# unique ribotypes = 15
	LL-04	23-Feb-95	vicinity	16	3	matches w/ water ribotypes = 20%
	LL-05	23-Feb-95	vicinity	15	3	
pig	PB-01	23-Feb-95	vicinity	16	3	# isolates = 75
	PB-02	23-Feb-95	vicinity	16	7	
	PB-03	23-Feb-95	vicinity	12	3	# unique ribotypes = 26
	PB-04	23-Feb-95	vicinity	15	5	matches w/ water ribotypes = 19%
	PB-05	23-Feb-95	vicinity	16	8	
septage	HU-01	11-Jan-94	CVLS08	15	6	# isolates = 227
	HU-02	11-Jan-94	CVLS08	8	3	
Grouped	HU-03	11-Jan-94	CVLS08	12	7	# unique ribotypes = 72
samples	HU-04	11-Jan-94	CVLS08	12	3	matches w/ water ribotypes = 8%
are from	HU-05	11-Jan-94	CVLS08	10	3	
different	HU-06	14-Sep-94	vicinity	8	3	
locations	HU-07	14-Sep-94	vicinity	14	3	
within	HU-08	14-Sep-94	vicinity	13	7	
the same	HU-09	19-Oct-94	CVLS07	0	--	
septic tank.	HU-10	19-Oct-94	CVLS07	0	--	
	HU-11	19-Oct-94	CVLS07	0	--	
	HU-12	22-Oct-94	CVLS07	6	5	
	HU-13	22-Oct-94	CVLS07	9	3	
	HU-14	22-Oct-94	CVLS07	16	4	
	HU-15	1-Feb-95	vicinity	33	5	
	HU-16	1-Mar-95	CVLS08	8	7	
	HU-17	1-Mar-95	CVLS08	55	27	

[1] see Figure 2. [2] A "0" value indicates *E. coli* could not be isolated from the sample.

unusual to observe a significant difference in concentration between a sample and its duplicate. The two results are the same order of magnitude. The geometric mean for all samples taken at CVLS08 is 216 CFU/100 mL.

3.3 *Bacteria Isolates*

Sixteen *E. coli* isolates on average were obtained from both water and source type samples. Tables 1. and 2. show the number of isolates for each sample. If less than 16 isolates are indicated for a sample it is due to:

- low concentrations in water samples (although the count was calculated as 0 CFU/100 mL for CVLS01-08B, a much larger volume may have been filtered multiple times allowing for some colonies to be isolated)
- low concentrations in source type samples due to the nature of an individual's intestinal flora, bacterial mix in a septic tank, or freshness of the sample
- sixteen or more isolates may have been obtained from a sample but some may not have tested positively as *E. coli*.

Part of the process of developing the MST methodology is to determine the optimum number of isolates to obtain from each sample type such that most, if not all, the ribotype diversity (all possible *E. coli* strains present) of the sample is represented. For this reason more than 16 isolates were obtained for some samples. This was done for water samples taken during the 18 February 1995 storm mentioned above. For locations other than CVLS04 (only one sample used) it was shown that obtaining a greater number of isolates did not increase the number of unique ribotypes observed. The isolate totals for these locations were adjusted for subsequent analyses to avoid overrepresentation. The adjusted totals are indicated in parentheses in Table 1.

A total of 1730 isolates was processed to the ribotyping stage. Nine of these were determined not to be *E. coli* according to the observed ribotypes. Seven needed reanalysis. This resulted in 1714 isolates available for the matching analysis. Of these 1714 isolates, 664 were water isolates with an adjusted total of 589. The remaining 1050 isolates were source type isolates, 227 isolated from septage and 823 isolated from animals. No isolates were obtained from fish suggesting they probably do not contribute to fecal coliform counts in the stream.

3.4 *Ribotypes and Ribotype Diversity*

Tables 1. and 2. present the number of different ribotypes observed for the total number of isolates obtained from each sample. The value for the number of unique ribotypes given in the last column in both tables refers to the number of different ribotypes observed for each sample type overall. For the 1714 isolates analyzed there were 353 unique ribotypes and therefore 353 groups or strains of *E. coli* represented. The 664 (589) water isolates

represented 171 strains and the 1050 source type isolates represented 239 strains. Of the 171 strains, 57 were found in both water and source type samples. Table 5. presents seven cases where an identical ribotype was determined for two different source types. Six of these strains were also found in water.

Ribotype diversity is a measure of the relative number of different strains of *E. coli* found in a sample. Here diversity was expressed simply as a ratio of ribotypes to isolates. There are more complicated algorithms for expressing diversity. They tend to be appropriate for larger and more consistent sample sizes. Samples having isolate numbers at either extreme were excluded from the calculations presented in Tables 3. and 4. The average ribotype diversity increases from 0.39 ±0.03 at CVLS01 to 0.62 ±0.15 at CVLS08. Of the four sources represented by the highest number of samples and number of sampling locations, septage samples exhibit the greatest average diversity, 0.41 ±0.19, followed by cows at 0.32 ±0.12 and horses and dogs at 0.26 ±0.13 and 0.25 ±0.12 respectively. The coarseness of the measure is reflected in the high values for standard deviation.

3.5 *Water-to-Source Ribotype Matches*

The results of ribotype matching can be presented in two general formats. One is to consider the number or percentage of matches found among ribotypes. The other is to consider the number or percentage of total isolates associated with strains matched by ribotypes. The data are presented using both formats. There were sampling limitations (e.g. sample size) both inherent to this kind of study and unique to the Little Soos Creek survey that can cause bias in the data. For this reason, the reader is advised to form an impression of the data that is more qualitative rather than quantitative.

The last column of Table 2. shows what percent (%) of the total number of strains associated with a given source type was also found in water. The greatest values are observed for cows (33%), dogs (32%), and horses (36%). Of the 72 strains associated with septage samples only, 6 (8%) were also found in water. The value of 50% shown for goat is not comparable as it only represents 2 ribotypes or strains.

Table 6. gives the detailed distribution of water-to-source ribotype matches for each designated reach of Little Soos Creek. CVLS01 represents the origin. CVLS03 and CVLS03a are the upper reach from the origin to CVLS03. CVLS04, CVLS05, and CVLS06 are grouped as the middle reach. CVLS07 is the first lower reach and CVLS08 represents the second lower reach.

The following is information for the first lower reach, CVLS07. It is a verbal example for interpreting the tabular data. The 152 isolates obtained for this reach belong to 64 *E. coli* strains (as determined by ribotype), 30 of these strains are new to this reach. Of the total number of strains, 26 (41%) are also associated with sources, this includes 5 of the new strains. For example, 7 strains found in the stream at this location are also associated with cows. Of these strains, 2 were observed uniquely in this reach. These 7 matches are

Table 3. Ribotype-to-isolate ratios as a coarse measure of diversity among water samples and locations.

Sample Number	Number of Isolates	Number of Ribotypes	Ribotype/ Isolate	Mean and Standard Deviation total	base	storm
CVLS01-05B	10	4	0.40	0.39	0.40	0.39
CVLS01-07S	14	5	0.36	0.03		0.05
CVLS01-10S	19	8	0.42			
CVLS03-01B	16	10	0.63	0.58	0.62	0.54
CVLS03-02S	16	6	0.38	0.15	0.15	0.16
CVLS03-03B	16	11	0.69			
CVLS03-04S	16	11	0.69			
CVLS03-05B	16	12	0.75			
CVLS03-06S	16	9	0.56			
CVLS03-07B	15	6	0.40			
CVLS07-01B	15	6	0.40	0.62	0.64	0.60
CVLS07-02S	16	9	0.56	0.16	0.20	0.13
CVLS07-03B	16	9	0.56			
CVLS07-04S	14	7	0.50			
CVLS07-05B	16	14	0.88			
CVLS07-06S	16	12	0.75			
CVLS07-09B	14	10	0.71			
CVLS08-02B	15	9	0.60	values for CVLS08	0.67	0.53
CVLS08-04S	16	8	0.50	and duplicate	0.15	0.13
CVLS08-05B	16	13	0.81			
CVLS08-07S	16	6	0.38	0.62		
CVLS08-08B	16	12	0.75	0.15		
CVLS08-10S	16	9	0.56			
CVLS08-11B	16	11	0.69			
CVLS08-14B	15	6	0.40			
duplicate of CVLS08						
CVDUPS-02B	16	9	0.56			
CVDUPS-04S	16	11	0.69			
CVDUPS-05B	16	14	0.88			
CVDUPS-08B	15	10	0.67			

Table 4. Ribotype-to-isolate ratios as a coarse measure of diversity among source samples and types.

Sample Number	Number of Isolates	Number of Ribotypes	Ribotype/ Isolate	Mean and Std. Dev.
CH-01	14	6	0.43	0.49
CH-02	13	7	0.54	0.06
CH-03	12	6	0.50	
CO-01	16	4	0.25	0.32
CO-02	16	6	0.38	0.12
CO-03	16	3	0.19	
CO-06	16	7	0.44	
CO-07	16	6	0.38	
CO-08	16	5	0.31	
CO-09	16	8	0.50	
CO-10	14	2	0.14	
DO-01	18	4	0.22	0.25
DO-04	16	4	0.25	0.12
DO-05	10	4	0.40	
DO-08	18	2	0.11	
DU-01	16	8	0.50	0.46
DU-02	14	6	0.43	0.05
HO-02	16	2	0.13	0.26
HO-03	14	6	0.43	0.13
HO-08	16	3	0.19	
HO-09	16	7	0.44	
HO-11	19	3	0.16	
HO-13	10	2	0.20	
HO-18	15	2	0.13	
HO-19	16	6	0.38	
HO-20	15	4	0.27	
LL-02	12	4	0.33	0.24
LL-03	16	4	0.25	0.07
LL-04	16	3	0.19	
LL-05	15	3	0.20	
PB-01	16	3	0.19	0.34
PB-02	16	7	0.44	0.13
PB-03	12	3	0.25	
PB-04	15	5	0.33	
PB-05	16	8	0.50	
septage				tank mean and std. dev.
HU-01	15	6	0.40	0.38
HU-02	8	3	0.38	0.13
HU-03	12	7	0.58	
HU-04	12	3	0.25	
HU-05	10	3	0.30	
HU-06	8	3	0.38	0.38
HU-07	14	3	0.21	0.16
HU-08	13	7	0.54	
HU-12	6	5	0.83	0.47
HU-13	9	3	0.33	0.32
HU-14	16	4	0.25	
HU-15	33	5	0.15	0.15
HU-16	8	7	0.88	0.68
HU-17	55	27	0.49	0.27
source mean for septage		0.41	std. dev.	0.19

Table 5. Cases of identical ribotypes appearing for two different source types.

Sample Types	# of Isolates Ratio
cow:deer	1:6
cow:septage	4:7
dog:horse	7:16
dog:horse	2:1
dog:horse	4:3
llama:septage	2:1
pig:septage	5:1

Table 6. Distribution of *Escherichia coli* in Little Soos Creek (matched to sources by ribotypes).

Stream Reach (See Figure [ ].)	# of stream ribotypes matched to sources	Source type matched	# of stream isolates represented	# of source isolates represented	Summary of stream to source matches
Origin [CVLS01]	1	chicken	1	5	ribotypes matched = 12 = 44% of total
	3	cow	14	52	
total # ribotypes = 27	2	dog	13	11	
	4	horse	15	71	total isolates matched = 45 = 63% of total
	1	llama	1	13	
	1	dog-horse	1	[2]	
total # isolates = 71	6	other stream reach	14	--	
	9	unique to reach	12	--	
Upper Reach [CVLS03, 3a]	3(1)[1]	chicken	5	11	ribotypes matched = 32 = 52% of total new ribotypes matched = 24 = 50% of total new
	7(2)	cow	23	54	
total # ribotypes = 61 new ribotypes = 48	5	dog	20	50	
	1	goat	2	7	
	7(1)	horse	16	93	
	1(1)	llama	1	3	
	2	pig	2	2	total isolates matched = 75 = 59% of total
	4(1)	septage	4	4	
total # isolates = 127	1	dog-horse	1	[2]	
	1	pig-septage	1	[2]	
	12	other stream reach	33	--	
	15	unique to reach	19	--	
Mid Reach [CVLS04, 5, 6]	4	cow	26	65	ribotypes matched = 20 = 51% of total new ribotypes matched = 8 = 38% of total new
	5(1)	dog	29	31	
	6(4)	horse	9	39	
total # ribotypes = 39 new ribotypes = 21	1(1)	llama	1	1	
	1	septage	8	1	
	1	cow-septage	1	[2]	
	1	dog-horse	3	[2]	total isolates matched = 79 = 80% of total
	1	pig-septage	2	[2]	
total # isolates = 99	9	other stream reach	10	--	
	10	unique to reach	10	--	
Lower Reach [CVLS07]	1	chicken	2	5	ribotypes matched = 26 = 41% of total new ribotypes matched = 5 = 17% of total new
	7(2)	cow	36	72	
	5	dog	37	44	
total # ribotypes = 64 new ribotypes = 30	6(1)	horse	11	37	
	3(2)	pig	3	5	
	1	septage	1	1	
	2	dog-horse	3	[2]	total isolates matched = 97 = 64% of total
	1	pig-septage	4	[2]	
total # isolates = 152	16	other stream reach	29	--	
	22	unique to reach	26	--	
Lower Reach [CVLS08]	1	chicken	2	5	ribotypes matched = 28 = 38% of total new ribotypes matched = 8 = 18% of total new
	7(3)	cow	41	76	
	5	dog	61	44	
total # ribotypes = 73 new ribotypes = 45	1	goat	1	7	
	6(2)	horse	16	62	
	1	llama	2	13	
	2(1)	pig	3	2	total isolates matched = 134 = 62% of total
	3(2)	septage	6	8	
total # isolates = 215	1	dog-horse	1	[2]	
	1	llama-septage	1	[2]	
	9	other stream reach	23	--	
	36	unique to reach	58	--	
Stream Totals	3	chicken	10	11	ribotypes matched = 57 = 33% of total
	13	cow	141	78	
	7	dog	152	48	
total # ribotypes = 171	1	goat	3	7	
	14	horse	64	108	total isolates matched = 421 = 63% of total = 71% of adjusted total
	3	llama	5	20	
total # isolates = 664 adjusted total = 589 (see text)	5	pig	8	7	
	6	septage	20	11	
	1	cow-septage	1	[2]	
	2	dog-horse	9	[2]	
	1	llama-septage	1	[2]	
	1	pig-septage	7	[2]	
	114	unique to stream	243	--	

[1] ( ) indicates number of water-source matches unique to stream reach for each source type. [2] See Table 5.

represented by 36 stream isolates and 72 cow isolates. The 26 matches made represent 97 of 152 (64%) isolates obtained for this reach. Of 64 total strains, 38 (55 isolates) were not associated with known source strains. Of these, 16 strains (29 isolates) were observed at other stream locations and 22 strains (26 isolates) were unique to this reach.

Table 7. provides a summary of the detailed information given in Table 6. Percent total ribotypes matched and the percent of total water isolates belonging to a matched strain are given for each reach. These percentages are then itemized according to source type. The actual ratios used to generate the percentages are given in parentheses as a reference. For example, out of a total of 171 different strains (as determined by ribotype) found in the stream overall, 57 (33%) were associated with known source strains. Of these strains, 114 (67%) are still unidentified to a source. Of the 57 (33%), 3 (5)% were associated with chickens, 13 (23%) with cows, 7 (12%) with dogs, 1 (2%) with goats, 14 (25%) with horses, 3 (5%) with llamas, 5 (9%) with pigs, 6 (11%) with septage, and 5 (8%) with ribotypes that are identical for two source types. Of the 589 water isolates, 421 (71%) belonged to a matched strain and 168 (29%) did not. Of the 421 (71%), 10 (2%) belong to strains associated with chickens, 141 (33%) with cows, 152 (36%) with dogs, 3 (1%) with goats, 64 (15%) with horses, 5 (1%) with llamas, 8 (2%) with pigs, 20 (5%) with septage, and 18 (5%) with strains found to be identical for two source types.

Table 7. Summary of water-to-source ribotype and isolate matches for Little Soos Creek.

Sample Type		Origin		Upper reach		Mid reach		Lower reach--1		Lower reach--2		Stream total	
Percent (%) Total Ribotypes Matched for Each Stream Reach													
matched water ribotypes		44	(12/27)	52	(32/61)	51	(20/39)	41	(26/64)	38	(28/73)	33	(57/171)
unmatched water ribotypes		56	(15/27)	48	(29/61)	49	(19/39)	59	(38/64)	62	(45/73)	67	(114/171)
Percent (%) Total Water Isolates Belonging to a Matched Strain													
matched water isolates		63	(45/71)	59	(75/127)	80	(79/99)	64	(97/152)	62	(134/215)	71	(421/589)
unmatched water isolates		37	(26/71)	41	(52/127)	20	(20/99)	36	(55/152)	38	(81/215)	29	(168/589)
% Contribution of Sources to Total Ribotypes and Isolates Matched at Each Reach													
chicken	(ribotypes)	8	(1/12)	9	(3/32)	0		4	(1/26)	4	(1/28)	5	(3/57)
chicken	(isolates)	2	(1/45)	7	(5/75)	0		2	(2/97)	1	(2/134)	2	(10/421)
cow	(ribotypes)	25	(3/12)	22	(7/32)	20	(4/20)	27	(7/26)	25	(7/28)	23	(13/57)
cow	(isolates)	31	(14/45)	31	(23/75)	33	(26/79)	37	(36/97)	31	(41/134)	33	(141/421)
dog	(ribotypes)	17	(2/12)	16	(5/32)	25	(5/20)	19	(5/26)	18	(5/28)	12	(7/57)
dog	(isolates)	29	(13/45)	27	(20/75)	37	(29/79)	38	(37/97)	46	(61/134)	36	(152/421)
goat	(ribotypes)	0		3	(1/32)	0		0		4	(1/28)	2	(1/57)
goat	(isolates)	0		3	(2/75)	0		0		1	(1/134)	1	(3/421)
horse	(ribotypes)	33	(4/12)	22	(7/32)	30	(6/20)	23	(6/26)	21	(6/28)	25	(14/57)
horse	(isolates)	33	(15/45)	21	(16/75)	11	(9/79)	11	(11/97)	12	(16/134)	15	(64/421)
llama	(ribotypes)	8	(1/12)	3	(1/32)	5	(1/20)	0		4	(1/28)	5	(3/57)
llama	(isolates)	2	(1/45)	1	(1/75)	1	(1/79)	0		1	(2/134)	1	(5/421)
pig	(ribotypes)	0		6	(2/32)	0		12	(3/26)	7	(2/28)	9	(5/57)
pig	(isolates)	0		3	(2/75)	0		3	(3/97)	2	(3/134)	2	(8/421)
septage	(ribotypes)	0		13	(4/32)	5	(1/20)	4	(1/26)	11	(3/28)	11	(6/57)
septage	(isolates)	0		5	(4/75)	10	(8/79)	1	(1/97)	4	(6/134)	5	(20/421)
cow-septage	(ribotypes)	0		0		5	(1/20)	0		0		2	(1/57)
cow-septage	(isolates)	0		0		1	(1/79)	0		0		0	(1/421)
dog-horse	(ribotypes)	8	(1/12)	3	(1/32)	5	(1/20)	8	(2/26)	4	(1/28)	4	(2/57)
dog-horse	(isolates)	2	(1/45)	1	(1/75)	4	(3/79)	3	(3/97)	1	(1/134)	2	(9/421)
llama-septage	(ribotypes)	0		0		0		0		4	(1/28)	2	(1/57)
llama-septage	(isolates)	0		0		0		0		1	(1/134)	0	(1/421)
pig-septage	(ribotypes)	0		3	(1/32)	5	(1/20)	4	(1/26)	0		2	(1/57)
pig-septage	(isolates)	0		1	(1/75)	3	(2/79)	4	(4/97)	0		2	(7/421)

Note: Any discrepancies in percentage totals are due to rounding error.

4.0 DISCUSSION

4.1 *Ribotype Diversity*

Although the ribotype to isolate ratio is a coarse measure of diversity, it does appear to support what intuitively would be expected in the stream. Not only the magnitude of contamination increases downstream as indicated by fecal coliform counts, but the complexity or numbers of contributing sources also increases. In this regard, the stream itself and its tributaries (e.g. CVLS03a) are viewed as potential sources of contamination—if a strain persists from one location to the next, the upstream location may be a source for the downstream location.

When considered for base and storm flows individually, the ribotype diversity is greater for base flow at all locations. The difference may not be significant given the large standard deviations. If so, the increase in contamination seen during storms can be said to represent more of the same contributing sources rather than additional sources. If the difference is significant, perhaps the greater base flow diversity is explained by a larger number of animals directly accessing the stream during fair weather. Additional samples are probably needed to determine if there is a significant difference in ribotype diversity during base and storm flows for Little Soos Creek.

For animal source types, the greatest diversity was calculated for chicken (3 samples) and duck (2 samples). Because of their small sample size, it may be incorrect to include these source types in a comparison with other source types having greater sample sizes. Of the remaining animal source types cow (0.32 ±0.12) and pig (0.34 ±0.13) exhibit the greatest diversity. The diversity observed for pigs may reflect a greater number of transient and resident strains present as a result of the rooting behavior of these animals or their tendency to have a diverse diet. Additional samples are probably needed to more fully assess the ribotype diversity of source types.

The highest diversity (other than for chicken and duck) was calculated for septage (0.41 ±0.19). It is also difficult to compare this number to the other source types since septage samples had less consistent isolate numbers per sample. This occurred because some samples were taken at a specific location within the tank before the layers were mixed and other samples were composites (more isolates obtained for these samples). Also, it was difficult to isolate *E. coli* from some septage samples where other bacterial species dominated. A more in depth study of the bacterial assemblages within septic tanks is necessary to gain a better understanding of the apparent complexity.

4.2 *Matching Efficiency*

Matching efficiency refers to the extent that MST is able to identify potential sources of contamination at a specific location. This is highly dependent on the source data available as mentioned above. There are two aspects of matching efficiency:

- the effectiveness of identifying strains found in water with strains found in sources by matching their ribotypes—percent of total water *ribotypes* matched.
- the effectiveness of the method to identify the sources of those strains present at the greatest frequency—percent of total water *isolates* matched.

For example, source matches were made for 57 of 171 (33%) total strains observed in water samples analyzed from Little Soos Creek. This may seem a low matching efficiency, however, 421 of 589 (71%) of all water isolates obtained from the stream belong to the 57 identified strains. Thus, the greater proportion (66%) of unidentified strains represents a smaller proportion (29%) of isolates. The unidentified strains are potentially contributing less to contamination. Substantial source control could be obtained by addressing those sources contributing the most to the 71% of contamination. The cost of substantially increasing the ribotype matching efficiency by additional sample processing may outweigh additional source control benefits achieved. This is especially true if the unidentified strains are rare and require more intensive source sampling. Therefore, when the two aspects of efficiency are considered together, the matching efficiency of MST as applied to Little Soos Creek is very good.

4.3 *Ribotype Confirmation and Database Development*

As mentioned, the ability of MST to track contamination is only as good as the information in the database. It is important to confirm and edit ribotypes as they are assigned and matches as they are made. This is done for isolates within each study and between studies. Out of this expansion and refinement process is developed a regional clonal database consisting of autoradiograms from individual isolates considered models of the strain they represent. These *type strains* are then used as standard measures to aid in assigning appropriate ribotypes to future isolates.

The whole process of confirmation is necessary to increase the degree of certainty involved in making matches. It is also integral to the process of understanding the diversity of the *E. coli* population in a region. This process involves exploration of such questions as:

- what level of uniqueness does a given strain have, i.e. is it a good indicator at the level of individual, species, genus, family, order, and so on—or not at all.
- if a strain is a good indicator, what is its range, i.e. is it appropriate for use at a specific location, within a watershed, or within a larger defined region.

- how long is a given strain genetically stable and physically persistent in a given source type such that it can continue to be used as an appropriate indicator.

Approaching the answers to these questions provides a greater understanding necessary for more effective use of MST by improving the ability to interpret the information MST provides. A more accurate interpretation of MST information also requires a reasonably thorough knowledge of the watershed including hydrology and locations and types of potential sources. A higher level of accuracy supports a more effective source control effort. An example where this type of information is helpful in reducing uncertainty is given below.

Table 7. indicates that sources contributing to the low levels of contamination observed for CVLS01 (located within the protected area for Lake Youngs) are chicken, cow, dog, horse, and llama. The presence of horse and dog strains at this location is highly possible given the wandering nature of dogs and the presence of a horse trail around the outer perimeter of the fence protecting the watershed. Although chicken and llama (each represented by only one strain, Table 6.) and cows (represented by three strains) are unlikely sources in the watershed for this location, there is a possibility that strains found in these animals are found in related species. In other studies the same *E. coli* ribotypes have been found in chicken and Canada geese. This is also true of cows, elks, and deer. Geese and deer are present upstream of this location. Another consideration is that an *E. coli* strain found at this location may colonize a host downstream after its ingestion by the host via streamwater consumption. In this way, the host becomes a secondary source of the *E. coli* strain.

The confirmation process and greater understanding of the regional *E. coli* population can also aid in interpreting the matches presented in Table 5. Here groups of isolates from two different source types exhibit identical ribotypes. If a strain is found in two source types, the cow to septage match for example, is it a resident strain in the individuals (animals or septic tanks) sampled or is it transient. If it is transient in one source type, which one. If it is transient in both, is it dominant in another unidentified source type. If it is dominant in both, is it a good indicator at any level. The animal to septage matches in Table 5. are not surprising since it is probable for animals in close and frequent contact with humans, such as pets and some livestock, to share strains found in humans or their wastewater.

A number of the questions raised here may be appropriately explored by statistical analyses. A statistical approach is more applicable as the extent of sampling and consistency in sample size (number of individuals sampled for each source type and number of isolates obtained from each sample) increases.

4.4 *Source Contribution and Stream Distribution Mapping*

The nature of pollution in natural waters is dynamic. In any given system there are changes in the land and weather, fluctuations in the contribution of potential sources, and transformations of the pollutant in the water environment. In the case of fecal coliform bacteria, and the organisms they are intended to indicate, there are a myriad of factors affecting their presence and distribution in a stream location at any point in time. Because of this random and probable nature of biological contamination, a monthly grab sample considered alone may not provide for an accurate characterization of the problem. Therefore, all the water samples for one location were considered together when defining the most probable scenario for potential source contribution at a given site and in the stream overall.

There was no upstream to downstream correlation of matching efficiency in terms of number of *isolates* matched. However, excluding CVLS01 (44%), there was a decrease in matching efficiency in terms of *ribotypes* matched from CVLS03 (52%) to CVLS08 (38%). The nature of contamination can become more complex downstream because the stream itself contributes contamination from upstream. Since downstream locations are more likely to have higher ribotype to isolate ratios (ribotype diversity) (Table 3.), more matches are required to fully characterize the problem. The lower *ribotype* matching efficiency for CVLS01 is probably due to little representation of wild animals in the Little Soos Creek database.

The focus of source sampling was based on those sources determined likely to be contributing most to the problem, livestock and septic systems. Because of the apparent number of dogs that freely roam and are tied in yards near the stream, these animals were also considered potential significant contributors. Most of the livestock types observed in the watershed were represented in sampling. However, the sampling focus was on cows and horses. It was difficult to gain property owner permission to sample their septic tanks in the watershed. The focus was on septic systems in the lower portion of the watershed and located in the permeable outwash material. The high matching efficiency achieved for the Little Soos Creek survey affirms these sampling choices and underscores the importance of a careful sanitary survey of the watershed prior to sampling for MST.

Stream Origin–Control Location

A discussion of the identified contributing sources at CVLS01, the stream origin, is given above. No human sources were identified. Of the *E. coli* strains observed in the water at this site, 26 of 71 (37%) are unidentified. The proportion of isolates identified as chicken, cow, and llama may be considered *under-identified* if they are also associated with another bird species or deer as discussed above. Fecal coliform counts for this location do not indicate a contamination problem so the uncharacterized isolates may not be of concern. It is likely that many of the unidentified strains are associated with wild animals.

Primary Sources

Cows, dogs, and horses are the primary contributors of the identified portion of *E. coli* strains observed in the stream. This is the case both in terms of numbers of strains and isolates belonging to these strains (see Table 7.). The percentages given below refer to the proportion of stream *isolates* matched to a given source (Table 7.). This is a measure of the extent of contribution to fecal coliform concentrations. The percentages in parentheses refer to the proportion of stream *ribotypes* matched to a given source (Table 7.).

Cows exhibited a fairly consistent, 32% on average, contribution over the entire stream except at CVLS07 where a slight peak of 37% was observed. The overall stream contribution was 33% (23% of ribotypes). The contribution identified with dogs increased from upstream, 29%, to downstream, 46%. This is not surprising since residential density and the number of pets having access to the stream increases downstream. The overall stream contribution was 36% (12% of ribotypes). Horses exhibited a much greater contribution for the reach upstream of CVLS03—33% at CVLS01 and 21% at CVLS03—than they did at all sites downstream of CVLS03—an average of 12%. The overall stream contribution was 15% (25% of ribotypes).

A systematic survey of how many cows and horses are kept in the watershed has not been performed. However, observations are that horses are present on more properties and may be in greater numbers. An explanation for the greater contribution to contamination by cows over horses may be attributable to: different pasturing practices observed for the two types of animals, the potential that more cow pastures are located close to the stream, the possibility that some of the strains associated with cows may also be shared with other animals, or differences in the fecal matter of the two animal types. This last possibility refers to the consistency of cow and horse feces.

It was more difficult to isolate organisms from horse feces. Fresh horse feces tends to consist of a relatively low-moisture, undigested hay core covered by a thin, moist, greasy layer. There was greater success isolating organisms from this outer layer suggesting it contains the greatest proportion of the bacterial population. In an open pasture this outer layer dries quickly and bacteria are killed or injured by ultraviolet radiation greatly reducing the potential for viable organisms to be transported to the stream. Cow feces is more consistent throughout and tends to contain high concentrations of bacteria. There were no difficulties isolating organisms from fresh cow fecal samples. This suggests that for comparable quantities of cow and horse feces, the cow feces has a greater potential to contribute to contamination over time.

Septage

There was no contribution to contamination by septage indicated at the origin. This increased slightly at CVLS03, 5%, peaked mid-reach, 10%, and then decreased at CVLS08, 4%. This was unexpected since the greatest density of older septic systems in outwash material, the scenario suspected to have the greatest potential for contributing to the problem, is present between CVLS07 and CVLS08. The overall stream contribution

of septage was 5% (11% of ribotypes). The percentage of water ribotypes matched to septage at CVLS03 was 13%, 5% at mid-reach, and 11% at CVLS08. This suggests that, although there was less contribution by septage indicated at CVLS08, perhaps there are more septic systems contributing (or systems with more diverse bacterial populations) in this reach. The inverse could be suggested for the mid-reach.

Although septage is a contributor to fecal bacterial pollution in Little Soos Creek, it is not indicated as a major source. However, even low levels of contribution by septage suggest the potential for Little Soos Creek to harbor a number of human viral, bacterial, and parasitic pathogens associated with human sources. For this reason, further investigation of the contribution by septic systems and of human exposure (particularly children) to the stream may be warranted. It is possible that a portion of the unidentified water isolates are attributable to septage. Additional sampling of septic tanks in the watershed and vicinity or use of riobotype information from regional studies may provide additional water-to-septage matches.

Sources for Further Consideration

Deer and duck were the only source types sampled that exhibited no matches with water isolates. This is likely due to small sample sizes for these animals. Chickens, goats, llamas, and pigs are represented by higher sample sizes. These source types did not exhibit a major contribution to the identified *E. coli* isolates. This is likely due to low representation in the database and the fact they are not present in high numbers in the watershed. All of these source types along with septage could be considered further by the use of data from other studies or additional sampling in the watershed.

Unknown Sources

Limitations of the study did not allow for comprehensive sampling of all potential source types in the watershed. A significant portion of the 168 (29%) water isolates not associated with sources and a portion of potentially under-identified (see above) isolates may be attributable to unsampled source types. These include numerous wild animals and other domestic animals such as cats. These source types have been represented in other studies using MST. Source strains from these studies that are found to be regionally applicable can be used in this study to make potential additional matches or help confirm current matches.

5.0 CONCLUSIONS

The application of MST to Little Soos Creek demonstrates the method as an effective tool in characterizing the nature of fecal contamination in receiving waters. Conventional techniques identify the presence and measure the level of contamination in water but do not identify the origin of microbial contaminants. A technique such as MST is needed for assessing the public health risk involved in human contact with water exposed to these pollutants, and allows for more effective control and management of the sources of contamination.

The data resulting from an MST matching analysis can be presented in two general ways:

- by the number or percentage of matches found among ribotypes
- by the number or percentage of total isolates associated with strains matched by ribotypes.

Both of these aspects of matching and identification are significantly affected by sampling limitations. For this reason, it is most appropriate to use the data to form a qualitative understanding of the problem rather than interpreting the data as an exact quantitative analysis. This wholistic approach makes use of a reasonably thorough knowledge of the watershed, the water and source samples collected, and the dynamics of microbial populations in the environment.

MST as applied to Little Soos Creek exhibited a very good matching efficiency. The ability of the method to track contamination is only as good as the information in the database. The effectiveness of the method overall will increase as the regional database develops and is refined.

In the Little Soos Creek study 57 of 171 (33%) ribotypes obtained from *E. coli* isolated from water were matched to source types other than water. Also, 421 of 589 (71%) water isolates belonged to the strains represented by these matched ribotypes. This suggests that, for the time period and stream locations sampled, MST identified the sources of approximately three-fourths of the fecal coliform contamination.

The primary sources of contamination were determined to be cows, dogs, and horses. The greatest proportion of water-to-source ribotype matches were found to be water-to-cow and water-to-horse. However, the greatest proportion of water isolates belonging to strains associated with these matched ribotypes were water-to-cow and water-to-dog. Tnis suggests that cows and dogs were the greatest contributors overall to the identified portion of the stream fecal coliform contamination. An exception is that, out of these three source types, horses were shown to be the greatest contributor at the stream origin (where fecal coliform counts are not high).

Although septage was identified as a contributor to the contamination problem, it is not indicated as a major source. From a public health standpoint, however, even low levels of contribution by septage could be significant because of the potential presence of human pathogens. For this reason, further investigation of the contribution by septic systems and of human exposure (particularly children) to the stream may be beneficial.

The remaining unmatched ribotypes (66%) represent the smaller proportion of unidentified stream isolates (29%). A significant portion of water ribotypes not matched to sources may be attributable to unsampled source types. These include numerous wild animals and other domestic animals such as cats. These source types have been represented in other studies using MST. Source strains from these studies that are found to be regionally applicable can be used in this study to make potential additional matches or help confirm current matches.

Additional matches are required for a more complete characterization of fecal contamination in Little Soos Creek. However, current data provide sufficient information to:

- achieve significant contamination reduction through design and implementation of control measures targeting cows, dogs, and horses.
- support further investigation of the public health risk posed by septic systems along the stream.

6.0 RECOMMENDATIONS

The fecal bacterial contamination of Little Soos Creek could be addressed by efforts to:

- Encourage livestock owners to observe best management practices for pastures in general and particularly those with direct access to the stream and its tributaries. This involves fencing to restrict access, streamside vegetation effective at filtering pollutants, avoidance of overpasturing resulting in bare and/or compacted earth, collection and proper storage/disposal of animal wastes, and alternatives to direct stream watering of animals.

- Encourage dog owners to reduce the time their animals are allowed to freely roam unattended and make an effort to dispose of dog fecal material properly (away from streams). Dog owners who keep their animals in yards with direct access to the stream could be encouraged to tie the dogs away from the stream and its tributaries and provide for streamside vegetation.

- Further investigate the impact of on-site septic systems in the area of Little Soos Creek.

Further characterization of the fecal contamination in Little Soos Creek could be achieved as the regional database is developed and applied to this study. The cost of substantially increasing the ribotype matching efficiency of this study by additional sample processing from the Little Soos Creek watershed may outweigh additional achievable source control benefits. However, if necessary to better understand the problem, particularly if levels of contamination increase, additional sampling and analysis could be performed.

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